# MICROBIOLOGICAL METHOD FOR ASSESSINGTHE EFFICACY OF DISINFECTIONS TARGETING MYCOBACTERIA

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## Introduction

Microbiological control methods for assessing the efficacy of decontamination measures are important not only from a microbiological perspective but also because of economic reasons: saving energy costs, manual labor and the amount of disinfectants used; also, they allow for an optimization in scheduling the times of shelters' unavailability during the procedures. It is of particular importance to certify the absence of pathogenic germs following the mandatory final disinfections applied as a measure of controlling the spread of infectious diseases prior to repopulating a farm with healthy livestock.

The tests most commonly used to control the effectiveness of surface disinfections are the coliform bacteria test, the microbiological field test, as well as the staphylococcus and enterococcus tests /1;2;3/. These methods have a good sensitivity as an indicator of disinfections fighting the less resistant microbes, such as unsporulated bacteria and large viruses. For the disinfections applied in controlling tuberculosis and paratuberculosis, however, the above-mentioned methods yield unsure results as they are not specific for mycobacteria, which are germs that can be up to thousands of times more resistant to the chemicals used in disinfections when compared to other bacteria /6/. Mycobacteria's increased resistance to disinfectants is due to a higher lipid proportion (especially mycolic acid) as a constituent of their bacterial wall, and to the higher molecular weight of these lipids, unlike the case of most bacteria /7/.

In the light of these data, it has been considered necessary to imagine, experiment and develop a new means of microbiological detection that can be applied where the control of disinfection's efficacy is specifically targeting mycobacteria, such as the case of those epidemiological measures taken against the mycobacterioses. As a test-germ, a strain of *Mycobacterium fortuitum* (sin. *M. giae*, *M. minetti*, *M. ranae*) has been chosen given its resistance to disinfectants, which comes very close to that of pathogenic mycobacteria /4/. Also, *M. fortuitum* has the advantage of growing quicker than *M. tuberculosis* on culture

media, forming characteristic colonies on the *MacConkey* agar. The new assessment method for the efficacy of disinfection uses carrier test objects deliberately contaminated with a suspension of *M. fortuitum*. In order to check the effectiveness of decontamination measures applied in a livestock shelter, following the removal of infected animals and a thorough cleaning, the carrier-tests are placed on both horizontal and vertical surfaces to be disinfected. After the disinfection procedures are finished and when the required contact time is over, the carrier-tests are treated with a neutralizing solution (specific to the nature of the disinfectant substance being used) and are brought to the microbiological laboratory. The survival of *M. fortuitum* is researched through insemination and cultivation on proper agar. The success of disinfection is confirmed if the indicator microorganisms have been neutralized (do not form colonies in culture).

**Material and methods**

**Preparation of carrier-tests.** *The carrier-tests are small, rectangular (9x6.5 cm) wood plates. Natural wood has the advantage of being relatively resistant to the contact with both acid and alkaline solutions, solid enough to withstand deformation during sterilization by heat, and it does not adversely affect the viability of the indicator bacteria (M. fortuitum). On each plate, one side has a circular area marked off (5.5 cm in diameter). The plates are* undergoing autoclave sterilization, and are afterwards contaminated with a *M. fortuitum* suspension in gelatin 0.3%. Gelatin's role is to fix the germs on the holder and to protect the bacterial cells against the action of the disinfectant solution. The germ pellicle thus obtained presents structural characteristics and resistance features against the disinfectant substances that are similar to those of mycobacteria associated in the form of a biofilm /5;6/.

After being aired for drying, each wooden plate was introduced into a sterile plastic envelope endowed with a tight closing system. The carrier-tests prepared in this way were stored in the refrigerator at 4°C, and the microbial load was periodically determined in order to set a maximum extent for its use.

**Checking the carrier-tests.** *The density of living bacteria on the contaminated surface of each test object was determined in the day of preparation, 24 hours after that, then at one, two and nine months respectively after its first placing in the refrigerator.*

**Assessing the efficacy of disinfection in livestock shelters.** Our method of detection has been applied in three shelters, each belonging to a different farm in which tuberculosis had developed. After the first epidemiological measures (depopulation, mechanical and hydro-sanitary cleaning) were completed, in each shelter were placed five carrier-tests contaminated

with the suspension of *M. fortuitum*. The plates were affixed to both the floor and the walls with double-adhesive tape.

The disinfection with *Santim F* (a disinfectant made basically of formaldehyde and quaternary ammonia) solution 3% followed. After disinfection, each carrier-test underwent neutralization with *Tween 80* solution 4% and ammonia, and was subsequently introduced in a sterile *Petri* dish and brought to the laboratory to check the survival of indicating microorganisms. This was done by inseminating them on a culture agar (*MacConkey*, modified). Two of the carrier-tests were simply washed with water, without being exposed to the action of the disinfectant. Other microbiological tests were also performed as a parallel assessment of disinfection efficacy: the coliform bacteria test, the staphylococcus test, the enterococcus test, and the microbiological field test.

**Results and discussion**

The bacteriological examinations performed on the carrier-tests stored in the refrigerator, the decrease in the density of living germs follows the curve presented in figure 1. This decrease was more pronounced during the first two months, and then it was slower. However, even after nine months of refrigeration, the indicator germ (*M. fortuitum*) could still be isolated in about 21% of cases.

Regarding the opportunity offered by a practical use of these carrier-tests, our experiments have shown that the method is simple enough and secure. After disinfecting the shelters in which tuberculosis developed in cattle, the attempts of retrieving the mycobacteria from the carrier-tests exposed in that environment have failed, while on the carrier-test used as witness mycobacteria were still alive. These results prove that the disinfectant solution used, its concentration and the contact period were efficient, the indicating bacteria being destroyed. The results were also upheld by the other, usual microbiological tests (coliform bacteria test, staphylococcus test, enterococcus test, and the microbiological field test).

**Conclusion**

The carrier-tests method for assessing the efficacy of disinfections is very reliable. It offers a high safety margin in determining whether a final disinfection has succeeded or not, thus being an effective microbiological and epidemiological tool in fighting the contagious diseases, before discontinuing the quarantine measures. *M. fortuitum* is recommended as the indicator germ of choice for controlling the disinfection efficacy when tuberculosis and paratuberculosis are involved. The carrier-tests must be made by an authorized

microbiological laboratory, using a standardized technology, in order to insure a proper density of living bacteria as indicators.

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**Fig.1** The evolution of total count germs (CFU/cm$^2$) on the carrier-tests experimentally contaminated with *M. fortuitum* and stored at +4°C in two successive experiments (m = months).